Hematology – Biochemistry

(Numbering is according to the 3rd set of slides on the website)

ONLY EXTRA NOTES

Slide 2

- Total transport of $CO_2$ (90%) involves direct and indirect ways.

- The carbamino Hb formation is the direct binding of Hb with $CO_2$ . The beta chain has a higher affinity to bind $CO_2$ and the deoxy- form is more reactive as well. Notice the protons formed by this process.

- The protons formed by the bicarbonate mechanism are taken up by the Hb buffer system.

Slide 3

- In tissues, there are lower oxygen pressures as well as higher $CO_2$ tensions, thus the oxygen is unloaded and the $CO_2$ is loaded to the N- terminal of the Hb to be transported toward the lungs where there are lower $CO_2$ tensions and high oxygen pressures, thus the Hb releases the $CO_2$ there and is being loaded with oxygen again.

Slide 4

- We start from $CO_2$ resulted from metabolism in tissues (Krebs cycle). This $CO_2$ is hydrated (adding water) by the enzyme carbon anhydrase inside the RBCs and the result is carbonic acid. This molecule dissociates to form bicarbonate ions as well as protons. The bicarbonate ion is dissolved into the plasma (arterial plasma nearby the tissues). While this is being carried on, the oxygenated Hb reaches the tissues and is therefore unloaded (deoxygenation) to release its 4 oxygens (tissues have low oxygen pressures, high $CO_2$ counts as well as high proton concentrations (lactic acid formation also plays a role in this)). The deoxy- Hb then accepts those protons which result from carbonic acid dissociation and prevents a drop in pH from occurring because this deoxy- form is **more basic**. This buffer system ($Hb.2H^+$) is then transported toward the lungs where there are high oxygen pressures and low $CO_2$ counts. Here, the Hb is oxygenated again and as a result, it releases its protons because the oxygenated form is **more acidic**. The released protons in the lungs encounter bicarbonate ions which are always present in high concentration in plasma and enter the RBCs again to re-form carbonic acid which - in turn - is dehydrated by carbon anhydrase to give water and $CO_2$ again. This $CO_2$ is dissolved into the plasma, moves to the alveoli and is finally exhaled from the lungs.

- Notice the opposite steps running in the tissues & lungs (Bohr's effect vs. Haldane's effect).

Slide 5

- The increase in basicity upon deoxygenation is due to the increase in pKa of an N- terminal of some amino groups and some histidine residues.

- Explanation: as you know, the transformation from the T form into the R form results in breaking down some ionic bonds and forming others. Upon deoxygenation (T form), a negatively-charged aspartate residue becomes close and helps His 146 accepting protons. So, after His 146 becomes positive (imidazolium), it forms an ion-pair with aspartate and thus, that negative charge of aspartate is the reason behind the increased basicity of the deoxy- Hb. An increase in the pKa definitely happens upon deoxygenation (more basic).

Slide 6

- This is a summary of Bohr's effect. Isohydric shift is when the deoxy- Hb accepts protons in the tissues and releases them in lungs. This indirectly transports $CO_2$ from tissues toward lungs in the form of bicarbonate. On the other hand, when bicarbonate ions are dissolved from RBCs into the plasma nearby the tissues, they cause a charge imbalance. This charge imbalance has to be overcome by a chloride shift, so chloride ions move into the RBCs. When bicarbonate ions re-enter the RBCs nearby the lungs, the chloride ions exit again.

Slide 7

- Embryonic Hb (zeta epsilon or alpha epsilon) is also called **hemoglobin gower**. It is present 1-3 months of pregnancy.

- HbF appears 2-3 months of pregnancy and its concentrations start to decline 1 month before birth.

- Alpha-globin family of chains has its genes present on chromosome 16 while Beta-globin family of chains has them on chromosome 11. Alpha family includes zeta (embryonic Hb) and alpha. Beta family includes epsilon (embryonic Hb), gamma (HbF), delta ($HbA_2$) and beta.

- Embryonic, fetal and adult forms are called developmental.

Slide 8

- $HbA_2$ has alpha (of the alpha family) and delta (of the beta family) chains. It appears about 12 weeks after birth, constitutes about 2-5% of Hb and persists during lifetime.

- $HbA_{1c}$ is another minor component hemoglobin that does not have a specific genetic constitution, instead, it's formed by glycosylation of existed Hb. There are also $HbA_{1a}$ and $HbA_{1b}$ which are bound to G6P or Fructose -1,6- diphosphate.

Slide 9 (see the last year's version – slide 5)

- The normal concentration of $HbA_{1c}$ is 3-6%. It becomes high (9-11%) in diabetic patients because it is glycosylated in a non-enzymatic reaction that depends on concentrations of reactants. So, it's a very good measurement for diagnosis and following up patients with diabetes who need a continuous glucose monitoring (this hemoglobin's lifespan is equal to the RBC's (120 days), which provides a good long-termed measurement).

Slide 10+11 (last year – slide 6)

- Zeta (embryonic) chain disappears 3 months after onset of pregnancy and is replaced by alpha.

- Epsilon (embryonic) disappears 3 months after onset of pregnancy and is replaced by gamma (fetal). Gamma chain's concentration remains high and starts to decline 1-2 months before birth and definitely, about 1% of it remains 6 months after birth and persists during adult life. Delta starts appearing after birth immediately and persists during lifetime (2-5%).

Slide 12 (last year – slide 7)

- Notice that there are 2 alpha chains genes in the alpha family and 2 gamma (A+G) chain genes in the beta family.

- Abnormal Hb structure is either in alpha or beta chains.

- Insufficiencies are also either in alpha or beta chains (thalassemia).

Slide 13

- Solubility abnormalities result in the formation of insoluble Hb polymers.

- HbM results from abnormalities of oxidation states of ferrous.

- Unstable Hb results from abnormalities in the tertiary structure.

- High oxygen affinity causes erythrocytosis, while low affinities cause cyanosis. All result from defects in the quaternary structure.

- Regional changes: altered exterior (usually hydrophilic) residues are usually harmless unless they become insoluble like those examples.

- There is also HbSC (heterozygous form - both S and C - present in Africa but rarely).

Slide 14

- Altered active site results in the formation of HbM and entering of water instead of oxygen.

- PPT = precipitation.

- Adding proline for example causes interruption of helical structure formation, thus causing instability and precipitation.

Slide 15

- Points of contact role: those points are the contacting sites between different subunits through which the conformational changes (resulted from oxygenation) are being transmitted (cooperativity concept). So, if they become defected, the affinity is absolutely going to be altered.

Slide 17

- Memorize the important examples (S and C).

(Remaining slides are not covered in this lecture)